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Factors influencing the success of vaginal and laparoscopic artificial insemination in churra ewes: a field assay

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Abstract

Pregnancy rate following artificial insemination (AI) in sheep is variable depending on several factors. The Churra breed (milk breed of the North-West of Spain) yields lower fertility results compared with other local and European breeds (Manchega, Latxa, Merino, Lacaune, Sarde, etc.). In this work we studied the influence of many factors on the fertility of the Churra breed (insemination technique, year, farm, age, male, number of inseminations per ewe, lambing-insemination interval and technician), analyzing lambing data obtained after 44448 inseminations (39.67% cervical AI via vagina, AIV, and 60.33% intrauterine AI using laparoscopy, AIL) in a categorical model. The most important factors influencing fertility after AI were farm, year, season, AI technique, and technician. AIL showed significantly higher fertility results than AIV (44.89% versus 31.25%). Season significantly affected fertility in both cases, but differences were more evident in AIV. Fertility dropped 1.74% (AIV) and 2.07% (AIL) per year as the ewes aged. Finally, AI fertility decreased when the lambing-insemination interval was lower than 10 weeks.

Keywords: Sheep; Vaginal insemination; Laparoscopic insemination; Variation factors

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1. Introduction

The development of artificial insemination (AI) and the consequent genetic improvement of farm animals have led to a remarkable increase in the productivity of livestock. AI in sheep has been poorly implemented and is carried out mainly with chilled semen because of the low fertility results obtained when using frozen-thawed semen [1]. This is due to the high structural complexity of the ewe cervix [2,3], which prevents deep AI and reduces the efficiency of the technique. Nowadays, laparoscopic insemination is an alternative method for AI using frozen-thawed semen [4].

Several factors affect the result of AI. Among them, we can highlight ewe handling, farm system, environmental elements, health, physiological status of the ewes, etc. [5–9]. It is important to control these factors, taking into account their influence on the outcome of AI.

Churra is a milk breed found predominantly in northwest Spain. According to Ugarte et al. [10] there are 750,000 Churra ewes representing 17% of Spanish dairy sheep. From 1986 onwards a selection and genetic improvement program has been in progress in this breed, but lambing rates following AI are very low (see results in other breeds: [5,11–13]). This situation has impaired both the efficacy of the genetic improvement program and the profitability of the farms. The low conception rates obtained using vaginal AI (AIV) favoured the rapid spread of intra uterine laparoscopic AI (AIL), which had improved fertility results. The technique has been used in more than 90% of all AI carried out during 2000 [14].

Both techniques have been used simultaneously in the field since 1993, therefore enabling us to study them and compare the results. The objective of our work was the analysis of many factors other than the AI technique (farm, year, season, male, ewe age, reproductive handling, technician, etc.) affecting fertility results in Churra sheep, and the evaluation of their importance depending on the AI technique used (AIV or AIL).

2. Material and methods

2.1. Animals

This study was conducted in the Castile and León region (Northern Spain) on a total of 22,758 Churra ewes belonging to 31 farms inscribed in the selection and genetic improvement program of the Churra breed and supervised by ANCHE (National Association of Churra Breeders). The farms were chosen according to the degree of their collaboration and fiability (IA data management). The total number of studied inseminations was 44448, performed over 8 years (17631 AIV with chilled semen and 26817 AIL with frozen-thawed semen). This number represents 45% of the total number of AI carried out under the supervision of ANCHE in this period. Churra sheep are bred for both milk (127 liters/lactation, about 120 days) and meat production (“lechazo”: lambs of approximately 10 kg of live weight [15]). Farms therefore aim at three lambings every two years in a continuous lambing system (Fig. 1). The average number of lambings on the studied farms was 1.34/ewe/year.

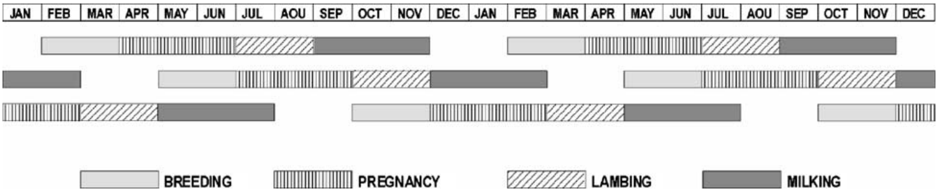

Fig. 1. Representation of the productive and reproductive events in Churra breed farms over two years.

2.2. Insemination procedures

Oestrus synchronization was carried out with fluorogestone acetate sponges (Chronogest, Intervet-Holanda), which were applied at 30 mg for multiparous ewes and 40 mg for nulliparous ewes. The time for the withdrawal of the sponges varied from 12 (multiparous ewes) to 14 (nulliparous ewes) days after their insertion. At sponge withdrawal, ewes were treated with 500 IU of PMSG (Folligon®, Intervet-The Netherlands). Multiparous females were inseminated at 55 $\pm$ 1 h for AIV and 54 $\pm$ 2 or 64 h $\pm$ 2 after pessary removal for AIL. Nulliparous females were inseminated only in AIL between 64 and 68 h after sponge withdrawal. These females were not inseminated via vaginal because the vaginal tract is not developed enough to use an ovine AI catheter.

Semen was obtained from 303 adult Churra rams of proven fertility, collected with an artificial vagina. Only ejaculates with the following characteristics were processed: volume $\geq$0.5 mL; sperm concentration $\geq 3 \times 10^9$ spz/ mL; mass motility (40×; 0–5) $\geq$4; total motility (200×) $\geq$75%. Insemination doses for AIV (ejaculates from the 241 rams) were diluted with a Tes-Tris-Fructose-egg yolk diluent at a concentration of 1600×10^6 spermatozoa/mL and put into French mini-straws (0.25 ml). Semen was chilled to 15 °C and held in a transportable refrigerator until arrival at the insemination farm (2–6 hours). For AIL, the semen (ejaculates from the 202 rams) was diluted to 100×10^6 spz/mL with Tes-Tris-Fructose extender containing 10% egg yolk and 4% glycerol, added in two steps [14]. The doses were packaged in 0.25 ml straws and frozen in a programmable biofreezer (Kryo 10-16 II, Planer™) using a rate of –20 °C/min down to –100 °C. The straws were kept in liquid nitrogen containers. Thawing was carried out in a water bath at 65 °C for six seconds. Thawed semen was used for AIL only when it complied with the following criteria: total motility $\geq$45% (Motility Analyzer 7.4G, Hamilton Thorn Research™), plasma membrane integrity >40% (cells positive to the hypo-osmotic swelling test—HOS test—in 100 mOsm/kg sodium citrate solution; automated evaluation by Coulter Counter™), and percentage of normal acrosomes >45% (evaluation with phase contrast microscopy, 600×).

AIV was performed by experienced technicians using a speculum with an attached light source and an ovine AI catheter (IMV™). Chilled semen (400×10^6 spermatozoa) was deposited in the entrance of the cervix. AIL was carried out by experienced technicians, as described by Evans and Maxwell [16], injecting 12.5×10^6 thawed spermatozoa (half a dose) into the lumen of each uterine horn.

Four technicians working for ANCHE took part in the project. Three of them were in charge of AIV. The other one, along with two technicians from the University of León, formed the group of inseminators in charge of AIL.

2.3. Data and statistical analysis

Data were taken from the parturition register used by ANCHE, as follows: farm, insemination type, insemination date, operator, ewe code, ram code and parturition date. This survey includes AI carried out from 1990 (year in which the first genetic assessment of the Churra breed was performed) to 1997. The variable studied was fertility, expressed as successful lambing. We regarded an insemination as positive (successful) if parturition took place between 139 and 158 days later. It was considered negative (unsuccessful) if parturition took place after 158 days, or not at all. We also considered three periods depending on the photoperiod and temperature throughout the year: breeding season (P1: September–January); non-breeding season (P2: February–June); and breeding season with high temperatures (P3: July–August).

Since fertility follows a binomial distribution, we carried out an analysis of variance for categorical data (see [16]), estimated by the method of maximum likelihood (CATMOD procedure, SAS™), using the following model:

$$F_{ijklmnopqr} = \mu + T_i + E_j + Y_k + S_l + A_m + \mathrm{AIV}_n + \mathrm{IPI}_o + R_p + I(T)_{iq} + e_{ijklmnopqr}$$

where: $F_{ijklmnopqr}$ is the success of the insemination (yes/no); μ is the global fertility; T_i is the type of insemination, i = AIV or AIL; E_j is the farm; j = 1–31; Y_k is the year of insemination, k = 1990–1997; S_l is the season of insemination, l = P1, P2 or P3; A_m is the age of ewes, m = from 1.5 to 6.5 years, in intervals of 1 year (including two extra classes for <1.5 and ≥6.5 years); AIV_n is the cumulative number of AI per ewe, n = 1, 2, 3 or ≥4; IPI_o is the last lambing-AI interval; o = nulliparous, ≤10 weeks, 10–22 weeks, or >22 weeks; R_p: ram; p = 0–15. (0: groups of those rams used in <600 AI; 1–15: individual rams, used in >600 AI each); $I(T)_{iq}$: inseminator nested per type of insemination; i = AIL → q = 1, 2 or 3, i = AIV → q = 1, 2, 3 or 4. $e_{ijklmnopqr}$ is the residual error.

A chi-square test (FREQ procedure) was used to compare different classes for those factors that showed a significant effect.

In order to provide a more detailed explanation of fertility variation, each AI technique (AIV and AIL) was analyzed separately. Thus, we applied the same model, removing factor T (type of insemination).

3. Results

3.1. General results

The analysis of variance of the different statistical models used to explain the variation in fertility is shown in Table 1. Global AI fertility was 39.5% and 31.3% for AIV, and

Table 1
Variation factors of AI fertility in the Churra breed

Variation factor	Total AI (n = 44,448)	AIV (n = 17,631)	AIL (n = 26,817)
Insemination technique	***		
Farm	***	***	***
Year	***	***	***
Season	***	***	***
Age of ewe	***	**	***
Lambing-AI interval	***	***	***
Ram	***	***	***
Technician	***	***	***
Cumulative number of AI/ewe	N.S.	*	N.S.

N.S.: non significant ($P \geq 0.05$); significant effect ($^{*}P < 0.05$); very significant effect ($^{**}P < 0.01$); highly significant effect ($^{***}P < 0.001$).

44.9% for AIL (Table 2). Fertility of examined AI is very similar to this of total AI inseminated by ANCHE (Fig. 2). These values are very homogeneous in the period of evaluation.

All factors had a highly significant effect ($P < 0.001$) on fertility except the total number of AI. After studying the two insemination techniques separately we observed that the number of accumulative AI per ewe had a significant effect on AIV fertility ($P < 0.05$).

3.2. AI technique

The type of AI used had a great impact on fertility. The percentage of lambing after AIL was 13 points higher than after AIV (Table 2), despite using frozen semen in AIL and chilled semen (with higher numbers of spermatozoa) in AIV.

3.3. Farm effect

There were important differences in fertility between farms (Fig. 3) for both AIV (between 12% and 52%) and AIL (between 19% and 59%). In general, the farms with good fertility results following AIV also had good results with AIL, and vice versa.

3.4. Year and season effect

Fertility fluctuated significantly over the years (Fig. 4), especially in the case of AIL, which showed over 60% fertility during the first years, followed by fertility rates below

Table 2
Fertility according to AI technique

Type of insemination	Fertility (%)*	Lambing/total AI
Vaginal AI (AIV)	31.25	5510/17631
Laparoscopic AI (AIL)	44.89	12038/26817
Total	39.48	17548/44448

* AI techniques differ (χ^2, $P < 0.001$).

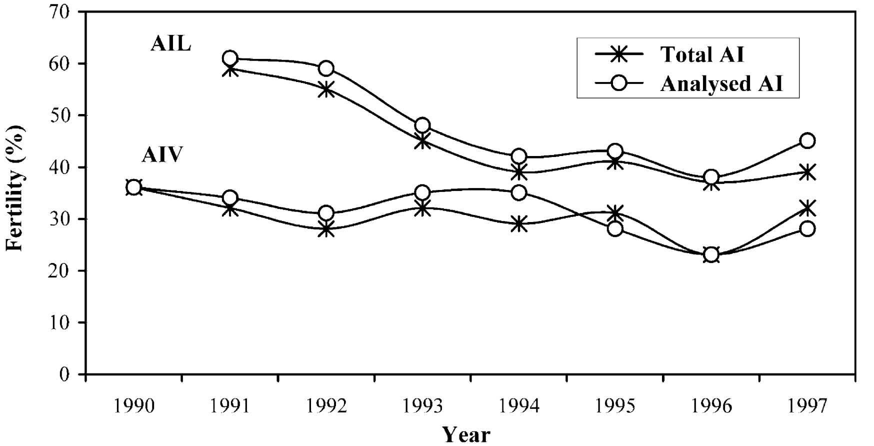

Fig. 2. AI fertility results in the studied sample and in the total population of sheep inseminated by ANCHE.

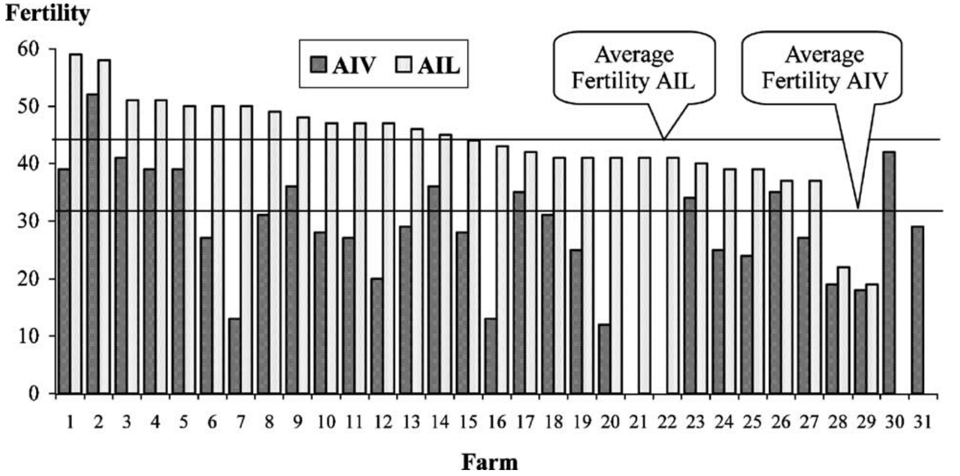

Fig. 3. Fertility after vaginal-cervical (AIV) and laparoscopic-uterine (AIL) insemination per farm (in farms 21, 22 and 31 only AIV or AIL were carried out).

50% during subsequent years (between 38% and 48%, from 1993). The lowest fertility rates for both techniques were recorded in 1996, due to severe droughts which affected both production and reproduction.

We found significant differences in AI fertility depending on the season (Table 3). The best fertility results were obtained in the September–January period of the breeding season, whereas lower results were obtained in the non-breeding season, particularly in the July–August period.

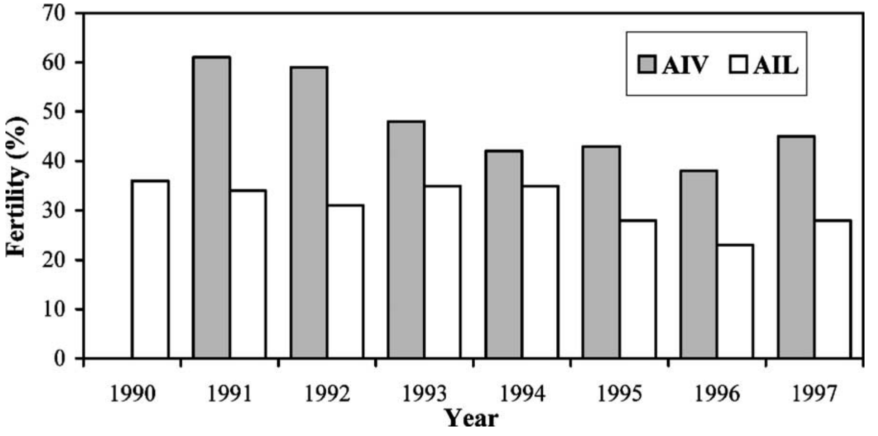

Fig. 4. Variation in fertility depending on year.

Table 3
Fertility according to season and AI technique

Season	% Fertility (Lambing /total AI)	
	AIV	AIL
P1 (September–January)	35.53[a] (1821/5125)	46.88[a] (5334/11379)
P2 (February–June)	29.79[b] (3566/11969)	43.96[b] (6077/13823)
P3 (July–August)	22.72[c] (122/537)	38.95[c] (629/1615)

In the same column, values with different superscripts differ ($P < 0.001$).

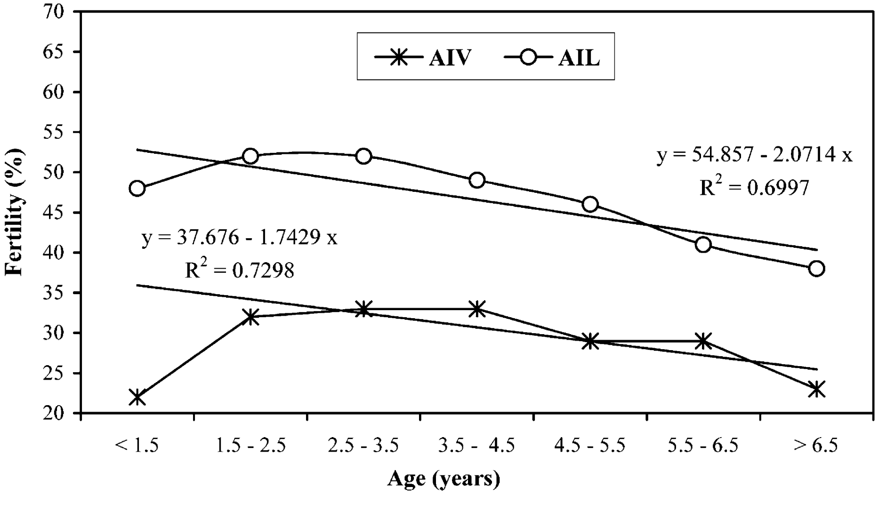

Fig. 5. Evolution of AI fertility results according to ewe age.

Table 4
Evolution of fertility depending on the number of lambings

Number of lambings	AIV % fertility (Lambing/total AI)	AIL % fertility (Lambing/total AI)
0		53.99[a] (2474/4582)
1	32.01[a] (1139/3558)	50.00[b] (2725/5450)
2	33.99[a] (883/2598)	48.01[bc] (1955/4072)
3	32.99[a] (613/1858)	45.99[cd] (1416/3079)
4	29.03[bc] (344/1185)	43.98[d] (903/2053)
5	26.07[c] (177/679)	38.96[e] (459/1178)
6	26.98[c] (99/367)	41.92[de] (231/551)
>6	24.07[c] (52/216)	36.00[e] (144/400)

In the same column, values with different superscripts differ (χ^2, $P < 0.05$).

3.5. Age and number of lambings

The age of the ewe significantly influenced fertility (Fig. 5), for both AIV ($P < 0.01$) and AIL ($P < 0.001$). After 1.5 years of age, the lambing rate decreased by 2.07% per year for AIL ($R^2 = 0.70$) and by 1.74% per year for AIV ($R^2 = 0.73$). The best fertility rates were recorded in ewes at between 1.5 and 4.5 years old; beyond this age, fertility declined remarkably. Fertility also decreased depending on the number of previous parturitions (Table 4). Due to the high correlation found among the age and number of lambings ($r = 0.85$; $P < 0.001$) only the former was included in the model, since both of them explained the same effect. Only 24% of the inseminated ewes were 4.5 years old or older (Fig. 6).

3.6. Number of cumulative AI per ewe

The number of cumulative AI per ewe did not have a significant influence on fertility. However, after analysing each technique of insemination separately, we noticed that this

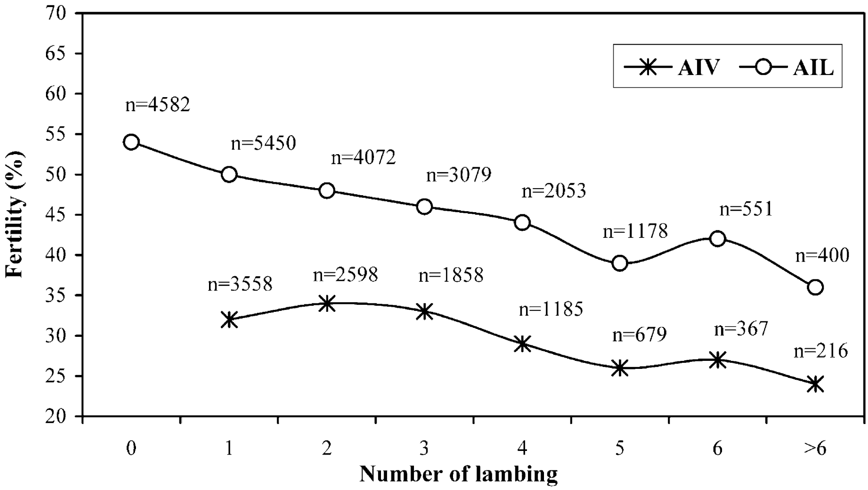

Fig. 6. Evolution of the fertility by number of lambing.

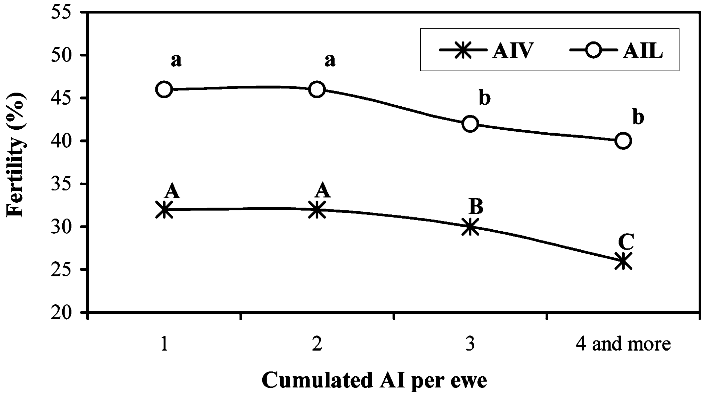

Fig. 7. Variation in fertility according to the number of cumulative AI per ewe. Different letters indicate significant differences (a, b: χ^2, $P < 0.01$; A, B, C: χ^2, $P < 0.05$).

factor acted in a significant way ($P < 0.05$) on fertility. Chi-square analysis showed that fertility decreased significantly with three or more cumulative AI for both AIV and AIL (Fig. 7).

3.7. Lambing-AI interval

Fertility decreased considerably when the lambing-AI interval was lower than 10 weeks (Fig. 8). Nulliparous females inseminated via AIL showed the highest fertility rates, demonstrating the effect of parturition on reproductive efficiency.

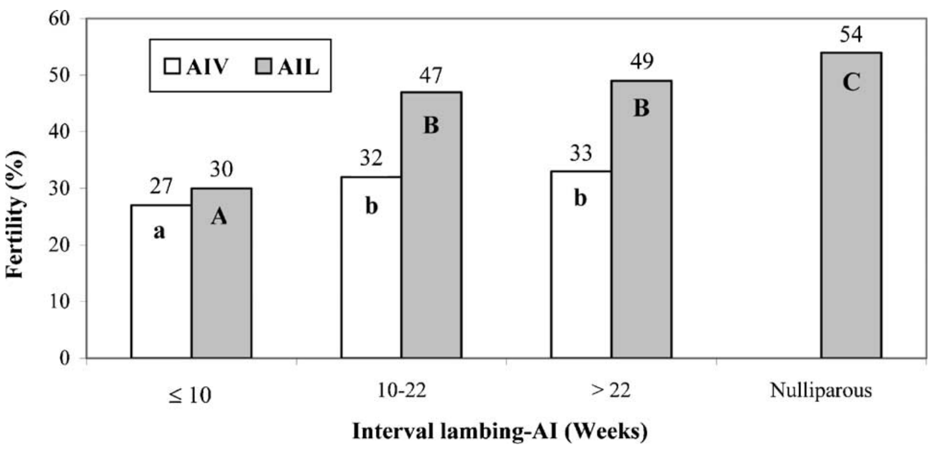

Fig. 8. Effect of the lambing-AI interval on AI fertility. Different letters indicate significant differences (a, b: χ^2, $P < 0{,}05$; A, B, C: χ^2, $P < 0.001$).

Table 5
AI fertility according to technician

Technician	% Fertility (Lambing/total AI)
AIV	
A	29.37^{a} (319/1086)
B	32.21^{b} (3446/10700)
C	43.16^{c} (641/1485)
D	23.85^{d} (598/2507)
AIL	
I	40.60^{A} (5556/13684)
II	49.26^{B} (2955/5999)
III	51.54^{C} (3237/6281)

Values with different superscripts a, b, c, d differ (χ^2, $P < 0.001$), Values with different superscripts A, B, C differ (χ^2, $P < 0.001$).

3.8. Ram effect

The male factor significantly influenced fertility results ($P < 0.001$) for both AIV and AIL. Despite the restrictions in the choice of ejaculates, we found important differences in fertility amongst rams, that was more pronounced in AIV (39%–57% for AIL, and 18%–45% for AIV).

3.9. Technician effect

Results of fertility varied significantly depending on the technicians ($P < 0.001$; Table 5) for both AIV and AIL.

4. Discussion

Fertility following AI in sheep depends on many factors, intrinsic and extrinsic to the inseminated female. These factors must be evaluated in order to improve AI results on farms. This study examines the effect of several factors on lambing rates following AI in Churra sheep.

AI techniques have been considered in many previous studies. According to several authors [17–20] AIL ensures significantly higher parturition rates than AIV, despite the fact that relatively lower numbers of frozen-thawed spermatozoa are used. This difference in fertility can be explained by the fact that the sheep cervix has a very high structural complexity, preventing deep AIV [2,3]. AIL allows this barrier to be bypassed, improving fertility even with lower quality frozen-thawed spermatozoa [1,21]. Besides the intrauterine placement of semen, AIL can also be simultaneously used to diagnose the reproductive tract, thus allowing those ewes with reproductive problems (underdeveloped or malformed genitalia—1.62% of the cases—, adhesions, ovarian pathologies, lack of response to estrus induction, early gestations) to be removed from the insemination lot. These problems would go unnoticed when performing AIV consequently impairing fertility results.

AIV fertility results in the Churra breed (31%) are different to those from other breeds [5,11–13,22] that which is due to multiple factors. Studies carried out in our laboratory [3] indicated that the cervix of the Churra breed has a peculiar morphology, characterized by a smaller size and more cervical folds than other breeds [2]. These differences imply greater difficulty in AIV and could explain, at least partially, the variability of AIV fertility between breeds.

Apart from the AI technique, our fertility results varied remarkably depending on the farm. Reproductive planning (intervals between lambings, season, age of first mating, AI technique, etc.) and animal handling (feeding, health, preparation of AI lots, etc.) have a great effect on fertility results [23–26]. Indeed, our results show that on 63% of the farms, AIV and AIL fertility results varied in the same way (both above or below the average values for all the farms) indicating that the conditions of each farm affected both techniques equally. Thus, in order to improve fertility results handling conditions on farms must be improved, together with a more widespread use of AI techniques.

Season was also very important in fertility. According to studies carried out on other breeds, changes in the photoperiod affect hormonal balance, causing seasonal reproductive variations [6,27]. We found that the effect of this variation is more important in AIV than in AIL. This fact highlights the importance of the insemination spot, since the semen is deposited closer to the actual site of fertilization of the ova in AIL, whereas it is deposited in the distal part of the vagina or in the proximal part of the cervix in AIV. In this case, other factors can affect fertility, such as cervical mucus quality and sperm transport. Some studies have demonstrated that progestagens alter cervical mucus characteristics, making it scarcer and more viscous, thus interfering with sperm transport in the cervix [28]. This could also happen due to the photoperiod. Moreover, high temperatures seemed to affect our results, suggesting that fertility will generally be significantly lower in midsummer.

Another important factor affecting fertility was ewe age. Our results coincide with those of other authors for different breeds, which predict 2–3% fertility reduction per year of age [5]. We noticed that females younger than 1.5 years showed lower fertility than older ewes, possibly due to the inclusion in this group of young ewes that had lambed previously and were undergoing nutritional shortage because of the high requirements for growth and lactation [29]. This effect was noticed especially in ewes that underwent AIV, since in this case this age class groups only primiparous females whose fertility was 10 percentual points lower than those of the next age class (1.5–2.5-year old). We did not notice this effect in the case of AIL because of the high proportion of nulliparous ewes in this group, which yielded relatively high fertility (54%). This dissimilar distribution of nulliparous ewes in this age class would have been responsible for the difference between AI techniques with regard the effect of age on fertility (Fig. 5).

Estrus syncronization implies hormonal treatment in ewes and this could have a negative effect on fertility. Our results indicated that fertility decreased with the number of previous insemination. In this sense, repeated application of PMSG might induce anti-PMSG antibodies in sheep and goat, rendering the affected females refractory to the treatment (in goat: [30]; in sheep: [31,32]).

The physiological status of the ewe clearly influences AI fertility. The lambing-AI interval had a great influence on fertility results, in accordance with the results of other authors [23,31,32]. The interval is related to the resting period of the reproductive apparatus of the ewe. Thus, the increasing reproductive rate imposed by the exigent production system implies short resting periods from lambing to AI, which affects fertility in a negative way. According to Boding et al. [33] reducing the lambing-AI interval to below 40–50 days induces a significant decrease in fertility, even after natural mating.

In conclusion, our data on AI in the Churra breed showed a great difference between AIV and AIL with regard to fertility. This difference justifies the use of AIL in many cases, since it reduces the detrimental influence of some sources of variation, which had a higher impact on AIV fertility. A large part of the variation in fertility was accounted for by the farm factor. Good handling practices and careful management of other sources of variation might help to prevent a low fertility outcome. When preparing ewe lots for AI, we recommend that ewes older than 5 years should not be used, that the lambing-AI intervals should be longer than 10 weeks and that insemination should not be carried out during high-temperature periods (July–August).

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